SPANDIDOS PUBLICATIONS

Antibody to hepatitis B core antigen is associated with the development of hepatocellular carcinoma in hepatitis C virus-infected persons: A 12-year prospective study

KAZUO TANAKA[1], YUMIKO NAGAO[1,2], TATSUYA IDE[1,2], RYUKICHI KUMASHIRO[1] and MICHIO SATA[1,2]

[1]Second Department of Medicine and [2]Department of Digestive Disease Information and Research, Kurume University School of Medicine, Asahi-machi, Kurume, Fukuoka 830-0011, Japan

Received December 9, 2005; Accepted January 26, 2006

Abstract. Several studies have reported that antibody to hepatitis B core antigen (anti-HBc) positivity may influence the development of hepatocellular carcinoma (HCC) in chronic hepatitis C patients, but the evidence is still not conclusive. In this study, we examined whether the presence of anti-HBc positive was associated with the development of HCC in hepatitis C virus (HCV)-infected subjects among the residents in an HCV hyperepidemic area who were followed up for 12 years. In an HCV hyperendemic area (positive rate of anti-HCV: 23.4%), 509 residents were examined by health screening in 1990. After 12 years of follow-up, we evaluated the risk factors for HCC. The incidence of HCC was compared between anti-HBc positive and anti-HBc negative subjects after 12 years of prospective observation. Univariate and multivariate analyses were conducted to determine risk factors for the development of HCC. The incidence of HCC was significantly higher in the anti-HBc positive group (13 subjects) than in the anti-HBc negative group (0 subjects) (P=0.012). Multivariate analysis identified positivity for anti-HBc and HCV RNA, history of icterus, and female gender as independent determinants of the development of HCC. Our findings provide clear evidence in a prospective study that presence of anti-HBc, that is, past hepatitis B virus (HBV) infection, is a risk factor for the development of HCC in HCV-infected people.

Introduction

The number of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection carriers worldwide is estimated at 350 million (1) and 170 million (2), respectively. HBV and HCV infections include substantial proportions of cases with past infection, asymptomatic carriers, acute hepatitis and chronic hepatitis, and HBV infections may cause fulminant hepatitis. Especially, chronic HBV and HCV infections may lead to cirrhosis and hepatocellular carcinoma (HCC) (1,3). It was reported that the frequency of HCC due to chronic HCV infection is higher in Japan than in any other country (4). Several studies have reported that occult HBV infection may also be one of the causative factors of HCC (5,6). The presence of occult HBV infection is diagnosed based on the fact that HBV DNA still exists in serum and liver tissue after hepatitis B surface antigen (HBsAg) disappears in acute or chronic HBV infection (7-9), or even after antiviral treatment is successful. Although some studies reported that occult HBV infection is associated with HCV-related liver dysfunction (10) or the development of HCC (11-13), these associations have still not been clearly demonstrated in a prospective study.

A higher incidence of HBV DNA is commonly seen in patients with anti-HBc-positive serology than in those with anti-HBc negative serology in coinfections with HBV and HCV (10), and using PCR amplification, most studies have demonstrated the presence of the HBV DNA genome in 22% to 87% of the patients who are HBsAg negative and HCV RNA positive (10,14-18). Some studies showed that HBV infection could occur in recipients of livers donated from subjects with anti-HBc but without HBsAg (19,20). That is, anti-HBc, which was initially considered to be an index for the past HBV infection in which all HBV had been cleared, has emerged as a convincing marker of occult hepatitis B (19,21-23). Also, several studies showed that the anti-HBc positivity was associated with the development of HCC in patients with HCV-associated chronic liver disease (11,24-26), but these associations have not been clearly demonstrated.

Since 1990, we have conducted health screenings of the residents of H town (adult population: 7,389), Fukuoka prefecture in northern Kyushu, Japan (27). This town is known for its high prevalence of liver disease. We previously reported that the town had a high prevalence of HCV carriers, 120/509 (23.6%) in 1990, and that HCV infection was the principal cause of liver dysfunction and HCC (27,28).

In the present study, we analyzed the influence of anti-HBc positivity on the development of HCC in HCV-infected people in the same town during 12 years in a prospective manner.

Correspondence to: Dr Kazuo Tanaka, Second Department of Internal Medicine, Kurume University School of Medicine, 67 Asahimachi, Kurume 830-0011, Japan
E-mail: wada.isi@alto.ocn.ne.jp

Key words: antibody to hepatitis B core antigen, occult hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, HCV hyper epidemic area

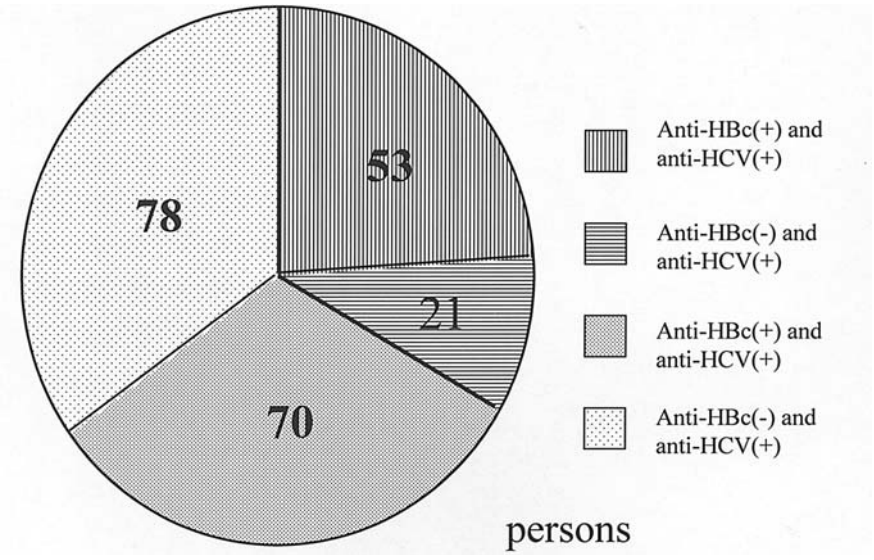

Figure 1. Diagram showing incident of hepatitis virus markers (anti-HCV and anti-HBc) among the 222 inhabitants 12 years ago. Fifty-three inhabitants were anti-HBc positive and anti-HCVpositive, 21 were anti-HBc negative and anti-HCVpositive, 70 were anti-HBc positive and anti-HCV negative, and 78 were anti-HBc negative and anti-HCV negative.

Subjects and methods

Subjects. In 1990, of a total 9,799 inhabitants, 739 (10%) of the 7,389 inhabitants >20 years old were randomly selected as follows: the names of the residents (as they appeared on their resident cards) were arranged in order according to the Japanese phonetic syllabary. Then every tenth resident was selected. As a result, 509 subjects (6.9% of H town residents) gave their informed consent to participate in the study.

Of 509 participants initially screened in 1990, 69 people had died and 55 people had moved to other regions as of 2002. Thus, 385 of the original 509 residents survived in the area and 139 residents agreed to participate in the medical follow-up survey, while 26 did not agree to participate, and the remaining 220 residents did not declare their intention either way in 2002. For 14 of these remaining 220 inhabitants, the records were obtained from the primary physicians. Consequently, we analyzed the outcome in terms of the liver disease in 222 inhabitants (69+139+14) in 2002.

Information on cigarette smoking, alcohol consumption, and history of icterus, and blood transfusion was obtained at the time of enrollment through interviews by the doctors in charge and experienced public health nurses. Smoking was defined as >10 cigarettes per day for >10 years. Alcohol consumption was defined as a daily intake of ≥75 g of ethanol per day for >10 years.

Serological assay. In 1990, sera were collected from all the participants, and conventional liver function tests were performed: serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transpeptidase (γ-GTP), total protein (TP), albumin (Alb), total cholesterol (TC), total bilirubin (TB), zinc turbidity (ZTT) were measured. Anti-HCV was measured using HCV PHA 2nd generation kits (Dainabot Co. Ltd., Tokyo, Japan). These results were confirmed using a second generation recombinant immunoblot assay (RIBA II) (Ortho Diagnostic System, Tokyo, Japan). Measurement of HBsAg and anti-HBc was performed with an enzyme immunoassay kit (Mizuho Medy Co. Ltd., Tosu, Saga, Japan). Titers of anti-HBc yielding >70% inhibition were assessed as positive.

Detection of HCV RNA by RT-PCR. All subjects who were anti-HCV-positive were tested for the presence of serum HCV RNA, which was detected by reverse transcription-nested polymerase chain reaction (RT-nested PCR) using primers based on the sequences of the 5'UTR (untranslated region) of the HCV genome, as described previously (29).

Statistical analysis. Continuous data were expressed as mean ± SD, minimum and maximum. Categorical data were expressed as frequency and/or percentage. For comparing the background between anti-HBc positive and negative, the χ^2 and Wilcoxon's test were used to analyze quantitative data. Univariate and multivariate analysis were performed by logistic regression to calculate odds ratio and its 95% confidence interval. The SAS (statistical analysis system) computer program (release 8.2; SAS Institute Inc., Cary, NC, USA) was used for the logistic regression. A P-value of <0.05 was considered statistically significant.

Results

In 2002, anti-HCV was detected in 74 of the 222 inhabitants (Fig. 1). HCV RNA was detected in 53 (71.6%), HBsAg in 1 (1.4%), and anti-HBc in 53 (71.6%) of these 74 people. We asked the primary physician of these 74 inhabitants about the diagnosis of liver disease, and found thereby that 8 inhabitants had died of HCC and 5 inhabitants had been treated for HCC (total 13 inhabitants).

The 74 inhabitants were divided into two groups: 53 who were positive and 21 who were negative for anti-HBc, and the clinical characteristics observed in the screening were compared between the two groups. No significant differences

haracteristics of anti-HCV positive patients with and without anti-HBc.

Characteristics	Anti-HBc positive (N=53)	Anti-HBc negative (N=21)	P-value
Age (year)	62.3±10.9	58.0±16.4	NS
Sex: M:F	23:30	05:16	NS
Smoking (%)	16 (30.2)	4 (19.0)	NS
History of icterus (%)	8 (15.1)	3 (14.3)	NS
Alcohol consumption (%)	3 (5.7)	2 (9.5)	NS
History of blood transfusion (%)	8 (15.1)	4 (19.0)	NS
ALT level (IU/l)	40.6±30.8	27.5±17.9	NS
HBsAg (%)	1 (1.9)	0 (0)	NS
HCV RNA (%)	39 (73.6)	14 (66.7)	NS
HCC (%)	13 (24.5)	0 (0)	0.012

Age and serum ALT level were expressed as mean ± SD. HCC, hepatocellular carcinoma; NS, not significant.

Table II. Univariate analysis of risk factors that influence the development of HCC.

Factors	HCC group (n=13)	non-HCC group (n=61)	Odds ratio	95% CI	P-value
Age (years)	65.3±8.1 (53-82)	60.1±13.4 (23-89)	1.035	0.984-1.088	0.1866
Sex: male (%)	6 (46.2)	22 (36.1)	0.658	0.196-2.205	0.4976
Smoking (%)	4 (30.8)	13 (21.3)	1.641	0.435-6.190	0.4646
Alcohol consumption (%)	5 (38.5)	22 (36.1)	1.108	0.323-3.804	0.8706
History of blood transfusion (%)	3 (23.1)	8 (13.1)	1.988	0.448-8.810	0.3659
History of icterus (%)	5 (38.5)	5 (8.2)	7.000	1.652-29.667	0.0083[a]
AST (IU/l)	65.5±31.1 (28-131)	33.0±21.9 (13-132)	1.041	1.015-1.068	0.0016[a]
ALT (IU/l)	57.5±24.8 (20-108)	32.6±27.1 (9-160)	1.028	1.006-1.050	0.0119[a]
γ-GTP (IU/l)	127.1±195.3 (17-720)	32.4±34.2 (7-196)	1.015	1.003-1.027	0.0158[a]
Total protein (IU/l)	7.97±0.88 (6.6-10.0)	8.05±0.58 (6.6-9.8)	0.808	0.309-2.107	0.6622
Albumin (g/dl)	3.98±0.49 (3.0-4.9)	4.33±0.31 (3.2-4.8)	0.094	0.017-0.507	0.0060[a]
Total cholesterol (mg/dl)	160.5±33.1 (111-224)	173.8±32.5 (111-257)	0.987	0.967-1.007	0.1851
Total bilirubin (mg/dl)	1.01±0.50 (0.5-2.3)	0.77±0.27 (0.4-1.3)	7.537	1.170-48.533	0.0335[a]
ZTT (KU)	15.35±5.76 (1.1-21.7)	11.40±4.86 (2.5-27.4)	1.161	1.026-1.314	0.0183[a]
Anti-HBc (%)	13 (100)	40 (65.6)	9.150	1.407-	0.0161[a]
HCV RNA (%)	13 (100)	40 (65.6)	9.150	1.407-	0.0161[a]

[a]P<0.05; HCC, hepatocellular carcinoma; CI, confidence interval. Age, AST, ALT, γ-GTP, total protein, albumin, total bilirubin and ZTT were expressed as mean ± SD (range).

were observed between the two groups in age, sex, smoking, history of icterus or blood transfusion, alcohol consumption, ALT level, HBsAg, or HCV RNA. Significant differences were observed for the incidence of HCC (13 versus 0) between these two groups (P=0.012) (Table I).

Univariate and multivariate analyses of factors that influenced the incidence of HCC. The influence of age, sex, smoking, history of icterus, history of blood transfusion, alcohol consumption, AST, ALT, γ-GTP, TP, Alb, TC, TB, ZTT, anti-HBc and HCV RNA on the development of HCC was analyzed by univariate and multivariate analyses.

Table II shows the basic characteristics of the 74 inhabitants with anti-HCV divided into two groups: a group with HCC (HCC group) and a non-HCC group, and shows the results of univariate analyses. The mean age and sex were not significantly different between the HCC group and non-HCC group. Serum levels of AST, ALT, γ-GTP, TB, and ZTT were significantly higher in the HCC group than in the non-HCC group (P<0.05). The serum level of Alb was significantly lower in the HCC group than in the non-HCC group (P<0.05). The frequency of anti-HBc, HCV RNA, and history of icterus were significantly higher in the HCC group than in the non-HCC group (P<0.05). The frequency of smoking, alcohol

Table III. Multivariate analysis of risk factors that influence the development of HCC.

Factors	Odds ratio	95% CI	P-value
Age (years)	0.987	0.852-1.132	0.8428
Sex: female	190.517	2.157- >999.999	0.0188[a]
Smoking	40.580	0.656- >999.999	0.0824
Alcohol consumption	5.051	0.163-3.804	0.3644
History of blood transfusion	0.964	<0.001- >999.999	0.9918
History of icterus	311.186	5.066- >999.999	0.0042[a]
AST (IU/l)	1.013	0.855-1.244	0.8776
ALT (IU/l)	0.974	0.791-1.101	0.7013
γ-GTP (IU/l)	1.006	0.990-1.080	0.6950
Total protein (IU/l)	15.131	0.227- >999.999	0.2035
Albumin (g/dl)	<0.001	<0.001-11.319	0.1236
Total cholesterol (mg/dl)	1.018	0.952-1.106	0.6028
Total bilirubin (mg/dl)	7.911	0.060- >999.999	0.4127
ZTT (KU)	0.695	0.370-1.196	0.1853
Anti-HBc positive	>999.999	1.556-	0.0292[a]
HCV RNA positive	>999.999	3.767-	0.0063[a]

[a]P<0.05; HCC, hepatocellular carcinoma; CI, confidence interval.

consumption, and history of blood transfusion were not significantly different between the HCC group and non-HCC group.

Multivariate logistic regression analyses identified anti-HBc positivity, HCV RNA positivity, history of icterus, and female sex as independent risk factors for the development of HCC (Table III).

Discussion

Several studies have shown that anti-HBc positivity was associated with the development of HCC in patients with HCV-associated chronic liver disease (11,24-26). However, considering the natural history of all HCV infections, the results of those previous studies have some problems, i.e., the observation period was short and the research was performed in a retrospective manner in patients with chronic hepatitis and liver cirrhosis. Our study was a prospective study that investigated the disease progress after 12 years, and was thought to reflect the natural history of HCV infections, because we did not investigate only HCV-associated chronic liver disease but also covered all HCV infections such as past HCV infection and asymptomatic carriers of HCV (30,31). In this study, we obtained clear evidence that anti-HBc-positivity was a risk factor for the development of HCC in HCV-infected people.

It has been suggested that HBV can induce liver tumor formation by at least two distinct mechanisms. First, HBV DNA sequences are frequently found integrated into chromosomes of hepatocytes that have evolved into HCC, and a direct role of HBV in hepatocarcinogenesis has thus been inferred (32,33). Second, HBV DNA sequences may be caused by disruption of tumor suppressor gene function (34). It has been shown that HBV DNA sequences can be detected in some of the liver or serum from anti-HBc-positive patients (9,10), and the presence of anti-HBc does not entirely exclude the possibility of chronic HBV infection. Though the presence of anti-HBc has been used as a marker of past HBV infection, the integration of HBV DNA in hepatocytes may cause carcinogenesis, as noted above. That is, anti-HBc-positivity may represent occult HBV infection. The presence of anti-HBc alone, in the absence of HBV DNA testing, has been used in some studies as a marker of occult hepatitis B (19,21-23). Pollicino *et al* provided clear evidence that occult HBV was a risk factor for the development of HCC and showed that the potential mechanisms whereby HBV might induce tumor formation occur in most cases of occult infection (6).

To detect occult HBV infection, it is necessary to examine whether HBV DNA is present. However, serum HBV DNA levels are frequently below the limits of detection in anti-HBc-positive patients, and there is a pronounced risk of false-positive results from contamination (35) or amplification of non-HBV-DNA targets, and the sensitivity of detection is variable (36,37). In a previous study in which serum HBV DNA was tested in 20 anti-HBc positive patients with HCV-associated HCC, HBV DNA was not detected by a real-time PCR assay with a minimum detection limit of $10^{1.7}$ copies/ml (1.7 log copies/ml) (38,39). Considering these results, it might not be possible to detect serum HBV DNA in some anti-HBc-positive subjects. Therefore, if we could examine liver tissues by PCR to examine whether occult HBV infection is present, we could be more certain of the presence of occult HBV infection.

In contrast to our findings, in some studies anti-HBc positivity was not found to be associated with the development

patients with HCV-associated chronic liver disease. One study showed that anti-HBc was detected significantly more frequently in blood donors with than without anti-HCV, but the prevalence of anti-HBc was no different between the patients with HCV-associated HCC and anti-HCV-positive blood donors. Therefore, no epidemiological evidence was obtained for a role of past HBV infection in hepatocarcinogenesis in patients infected with HCV in Japan (40). Also, Yano *et al* showed that the clinical features of HCV-associated HCC were unaffected by anti-HBc-positivity (39). In addition, a study in Taiwan suggested that occult HBV infection might have little influence on the clinicopathologic course of chronic HCV infection (9).

It was reported that the frequency of HCC due to chronic HCV infection is higher in Japan compared with any other country (4). If the frequency of HCC due to chronic HCV infection is high, it is necessary to consider the possibility that anti-HBc positivity may be associated with hepatocarcinogenesis. In addition to HBV, other environmental and host factors might also be associated with the pathogenesis of HCC (4,41-43).

We continued carrying out health screenings of the residents of H town and conducted a cohort study of liver disease among the same residents over a 12-year period. The results of this study showed that anti-HBc is associated with the development of HCC in HCV-infected people.

Acknowledgements

We would like to thank the following institutions for collection of data of the residents' liver disease: Amagi Asakura Medical Association Asakura Hospital, Ishii Surgery Clinic, Shigematsu Medical Office, Tanabe Medical Office, Haki Clinic, Fukuoka Prefectural Hepato-gastroenterological Center, Moriyama Medical Office, Yamaga Medical Office, Wada Surgery Clinic, Chikugo River Onsen Hospital, Harazuru Onsen Hospital, Digestive Tract Internal Medicine of St. Mary's Hospital, Digestive Tract Internal Medicine of Koga Hospital, and Majima Digestive Tract Clinic. Moreover, we thank the following companies for supplying the related inspection reagent kits for the hepatitis virus: Ortho-Clinical Diagnostics, K.K., Roche Diagnostics K.K., and Abbott Laboratories. This study was supported in part by grants as a project for establishing new high technology research centers, by a Grant-in-Aid for Scientific Research (C) (No. 11670548), by a Grant-in-Aid for Encouragement of Young Scientists (No. 14770256) from the Ministry of Education, Science, Sports and Culture of Japan, and the Hepatitis C Research Group (2001-2003) under the auspices of the Ministry of Health, Labor and Welfare.

References

1. Maynard JE: Hepatitis B: global importance and need for control. Vaccine (Suppl): S18-S20, 1990.
2. WHO: Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the viral hepatitis prevention board, Antwerp, Belgium. J Viral Hepat 6: 35-47, 1999.
3. Niederau C, Lange S, Heintges T, *et al*: Prognosis of chronic hepatitis C: result of a large, prospective cohort study. Hepatology 28: 1687-1695, 1998.
4. Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 62 (Suppl 1): 8-17, 2002.
5. Yotsuyanagi H, Shintani Y, Moriya K, *et al*: Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. J Infect Dis 181: 1920-1928, 2000.
6. Pollicino T, Squadrito G, Cerenzia G, *et al*: Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. Gastroenterology 126: 102-110, 2004.
7. Blackberg J and Kidd-Ljunggren K: Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation. J Hepatol 33: 992-997, 2000.
8. Fong T-L, Di Bisceglie AM, Gerber MA, *et al*: Persistence of hepatitis B. Hepatology 18: 1313-1318, 1993.
9. Kao JH, Chen PJ, Lai MY, *et al*: Occult hepatitis B virus infection and clinical outcomes of patients with chronic hepatitis C. J Clin Microbiol 40: 4068-4071, 2002.
10. Caccoiola I, PollicinoT, Squadrito G, *et al*: Occult hepatitis B virus infection in patient with chronic hepatitis C liver disease. N Engl Med 341: 22-26, 1999.
11. Sheu JC, Huang GT, Shin LN, *et al*: Hepatitis C and B viruses in hepatitis surface antigen-negative hepatocellular carcinoma. Gastroenterology 103: 1322-1327, 1992.
12. Paterlini P, Driss F, Nalpas B, *et al*: Persistence of hepatitis B and hepatitis C viral genomes in primary liver cancers from HBsAg-negative patients study of a low-endemic area. Hepatology 17: 20-29, 1993.
13. Kubo S, Nishiguchi S, Hirohashi K, *et al*: Clinical significance of prior hepatitis B virus infection in patients with chronic hepatitis C virus-related hepatocellular carcinoma. Cancer 86: 793-798, 1999.
14. Fukuda R, Ishimura N, Niigaki M, *et al*: Serologically silent hepatitis B virus confection in patients with chronic hepatitis C virus associated chronic liver disease: clinical and virological significance. J Med Virol 58: 201-207, 1999.
15. Uchida T, Kaneita Y, Gotoh K, *et al*: Hepatitis C virus is frequently coinfected with serum marker negative hepatitis B virus: probable replication promotion of the former by the latter as demonstrated by *in vitro* cotransfection. J Med Virol 52: 399-405, 1997.
16. Gonzalez S, Navas S, madejon A, *et al*: Hepatitis B and D genomes in hepatitis B surface antigen negative patients with chronic hepatitis C. J Med Virol 45: 168-173, 1995.
17. Villa E, Crottola A, Buttafoco P, *et al*: Evidence of hepatitis B virus in patients with chronic hepatitis C with or without serological markers of hepatitis B. Dig Dis Sci 40: 8-13, 1995.
18. Berger A, Doerr HW, Rabenau HF, *et al*: High frequency of HCV infection in individuals with isolated antibody to hepatitis B core antigen. Intervirology 43: 71-76, 2000.
19. Dickson RC, Everhart JE, Lake JR, *et al*: Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. Gastroenterology 113: 1668-1674, 1997.
20. Uemoto S, Sugiyama K, Marusawa H, *et al*: Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. Transplantation 65: 494-499, 1998.
21. Sagnelli E, Coppola N, Scolastico C, *et al*: HCV genotype and 'silent' HBV co-infection: two main risk factors for a more severe liver disease. J Med Virol 64: 350-355, 2001.
22. Joller-Jemelka J, Wicki A and Grob P: Detection of HBs antigen in anti-HBc alone positive sera. J Hepatol 21: 269-272, 1994.
23. Marusawa H, Umemoto S, Higikata, *et al*: Latent hepatitis B virus infection in healthy with antibodies to hepatitis B core antigen. Hepatology 31: 488-495, 2000.
24. Chiba T, Matsuzaki Y, Abei M, *et al*: Multivariate analysis of risk factors for hepatocellular carcinoma in patients with hepatitis C virus-related liver cirrhosis. J Gastroenterol 31: 552-558, 1996.
25. Okada S, Sato T, Okusaka T, *et al*: Past exposure to hepatitis B virus as a risk factor for hepatocellular carcinoma in patients with chronic liver disease. Br J Cancer 77: 2028-2031, 1998.
26. Marusawa H, Osaki Y, Kimura T, *et al*: High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related liver disease in Japan. Gut 45: 284-288, 1999.

27. Sata M, Nakano H, Suzuki H, *et al*: Sero-epidemilogic study of hepatitis C virus infection in Fukuoka, Japan. J Gastroenterol 33: 218-222, 1998.
28. Nagao Y, Fukuizumi K, Tanaka K, *et al*: The prognosis for life in an HCV hyperendemic area. Gastroenterology 125: 628-629, 2003.
29. Okamoto H, Okada S, Sugiyama Y, *et al*: Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5'-noncoding region. Jpn J Exp Med 60: 215-222, 1990.
30. Nagao Y, Tanaka K, Kobayashi K, *et al*: Analysis of approach to therapy for chronic liver disease in an HCV hyperendemic area of Japan. Hepatol Res 28: 30-35, 2004.
31. Nagao Y, Tanaka K, Kobayashi K, *et al*: A cohort study of chronic liver disease in an HCV hyperendemic area of Japan: a prospective analysis for 12 years. Int J Mol Med 13: 257-265, 2004.
32. Feitelson MA and Duan LX: Hepatitis B virus X antigen in the pathogenesis of chronic infections and development of hepatocellular carcinoma. Am J Pathol 150: 1141-1157, 1997.
33. Tamori A, Nishiguchi S, Kubo S, *et al*: Possible contribution to hepatocarcinogenesis of X transcript hepatitis B virus in Japanese patients with hepatitis C virus. Hepatology 29: 1429-1434, 1999.
34. Zhou YZ, Slagle BL, Donehower LA, *et al*: Structural analysis of a hepatitis B virus genome integrated into chromosome 17p of a human hepatocellular carcinoma. J Virol 62: 4224-4231, 1988.
35. Khristova M, Nainan O, Xia G-L, *et al*: False-positive HBVDNA results among persons with only antibody to hepatitis B core antigen. Antiviral Ther 5 (Suppl 1): B22, 2000.
36. Ke-Qin H: Occult hepatitis B virus infection and its clinical implications. J Viral Hepat 9: 243-257, 2002.
37. Torbenson and Thomas DL: Occult hepatitis B. Lancet Infect Dis 2: 479-486, 2002.
38. Ide T, Kumashiro R, Koga Y, *et al*: A real-time quantitative polymerase chain reaction method for hepatitis B virus in patients with chronic hepatitis B treated with lamivudine. Am J Gastroenterol 98: 2048-2051, 2003.
39. Yano Y, Yamashita F, Sumie S, *et al*: Clinical significance of antibody against hepatitis B virus core antigen in patients infected with hepatitis C virus-related hepatocellular carcinoma. Liver Int 23: 227-231, 2003.
40. Hiraoka T, Katayama K, Tanaka J, *et al*: Lack of epidemiological evidence for a role of resolved hepatitis B virus infection in hepatocarcinogenesis in patients infected with hepatitis C virus in Japan. Intervirology 46: 171-176, 2003.
41. Matsuzaki Y, Chiba T, Hadama T, *et al*: HBV genome integration and genetic instabillity in HBsAg-negative and anti-HCV-positive hepatocellular carcinoma in Japan. Cancer Lett 119: 53-61, 1997.
42. Matsuzaki Y, Sato M, Saito Y, *et al*: The role of previous infection of hepatitis B virus in HBs antigen negative and anti-HCV-positive Japanese patients with hepatocellular carcinoma: etiological and molecular biological study. J Exp Clin Cancer Res 158: 73-84, 1999.
43. Chen P-J and Chen D-S: Hepatitis B virus infection and hepatocellular carcinoma. Molecular genetics and clinical perspectives. Semin Liver Dis 19: 253-262, 1999.